

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07K 14/605, A61K 38/26	A1	(11) International Publication Number: WO 98/08872 (43) International Publication Date: 5 March 1998 (05.03.98)
(21) International Application Number: PCT/DK97/00360 (22) International Filing Date: 1 September 1997 (01.09.97) (30) Priority Data: 0931/96 30 August 1996 (30.08.96) DK 1259/96 8 November 1996 (08.11.96) DK (71) Applicant (for all designated States except US): NOVO NORDISK A/S [DK/DK]; Novo Allé, DK-2880 Bagsværd (DK). (72) Inventors; and (75) Inventors/Applicants (for US only): KNUDSEN, Liselotte, Bjerre [DK/DK]; Valby Langgade 49A, 1tv., DK-2500 Valby (DK). SØRENSEN, Per, Olaf [DK/DK]; Applebys Plads 27, 5. mf., DK-1411 Copenhagen K (DK). NIELSEN, Per, Franklin [DK/DK]; Dalsø Park 59, DK-3500 Værløse (DK). (74) Common Representative: NOVO NORDISK A/S; Corporate Patents, Novo Allé, DK-2880 Bagsværd (DK).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: GLP-2 DERIVATIVES (57) Abstract Derivatives of hGLP-2 and analogues thereof and fragments thereof and analogues of such fragments having a lipophilic substituent have interesting pharmacological properties, in particular they have a more protracted profile of action than the parent peptides.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

GLP-2 DERIVATIVES

FIELD OF THE INVENTION

- 5 The present invention relates to novel derivatives of human glucagon-like peptide-2 (hGLP-2) and analogues thereof and fragments thereof and analogues of such fragments which have a protracted profile of action and to methods of making and using them.

10 BACKGROUND OF THE INVENTION

Peptides are widely used in medical practice, and since they can be produced by recombinant DNA technology it can be expected that their importance will increase also in the years to come. When native peptides or analogues thereof are used in therapy it is
15 generally found that they have a high clearance. A high clearance of a therapeutic agent is inconvenient in cases where it is desired to maintain a high blood level thereof over a prolonged period of time since repeated administrations will then be necessary. Examples of peptides which have a high clearance are: ACTH, corticotropin-releasing factor, angiotensin, calcitonin, insulin, glucagon, glucagon-like peptide-1, glucagon-like peptide-2, insulin-like
20 growth factor-1, insulin-like growth factor-2, gastric inhibitory peptide, growth hormone-releasing factor, pituitary adenylate cyclase activating peptide, secretin, enterogastrin, somatostatin, somatotropin, somatomedin, parathyroid hormone, thrombopoietin, erythropoietin, hypothalamic releasing factors, prolactin, thyroid stimulating hormones, endorphins, enkephalins, vasopressin, oxytocin, opioids and analogues thereof, superoxide
25 dismutase, interferon, asparaginase, arginase, arginine deaminase, adenosine deaminase and ribonuclease. In some cases it is possible to influence the release profile of peptides by applying suitable pharmaceutical compositions, but this approach has various shortcomings and is not generally applicable.

- 30 The amino acid sequence of GLP-2 and other preproglucagon fragments is given *i.a.* by Schmidt *et al.* (*Diabetologia* 28 704-707 (1985)). Little is known about the physical chemical properties of GLP-2 but GLP-2 is expected, like GLP-1, to be a highly flexible and unstable molecule. GLP-2 and fragments thereof and analogues of GLP-2 and fragments thereof are

potentially useful *i.a.* in regulation of appetite and in the treatment of small bowel syndrome. However, the high clearance limits the usefulness of these compounds, and thus there still is a need for improvements in this field.

5

SUMMARY OF THE INVENTION

Preproglucagon, from which GLP-2 originates, is synthesized *i.a.* in the L-cells in the distal ileum, in the pancreas and in the brain. Processing of preproglucagon to give GLP-1 and
10 GLP-2 occurs mainly in the L-cells. GLP-2 is a 34 amino acid residue peptide. A simple system is used to describe fragments, analogues and derivatives of GLP-2. Thus, for example, Lys²⁰GLP-2(1-33) designates a fragment of GLP-2 formally derived from GLP-2 by deleting the amino acid residues No. 34 and substituting the naturally occurring amino acid residue in position 20 (Arg) by Lys. Similarly, Arg³⁰Lys³⁵(N^t-tetradecanoyl)GLP-1(1-35)
15 designates a derivative of a GLP-2 analogue formally derived from GLP-2 by C-terminal addition of a Lys residue, exchange of the naturally occurring amino acid residue in position 30 (Lys) with an Arg residue and tetradecanoylation of the ϵ -amino group of the Lys residue in position 35.

20 In its broadest aspect, the present invention relates to derivatives of GLP-2 and analogues thereof. The derivatives according to the invention have interesting pharmacological properties, in particular they have a more protracted profile of action than the parent peptides.

25 In the present text, unless otherwise specified, "GLP-2" designates human GLP-2. The designation "an analogue" is used to designate a peptide wherein one or more amino acid residues of the parent peptide have been substituted by another amino acid residue and/or wherein one or more amino acid residues of the parent peptide have been deleted and/or wherein one or more amino acid residues have been added to the parent peptide.

30

The term "derivative" is used in the present text to designate a peptide in which one or more of the amino acid residues have been chemically modified, e.g. by alkylation, acylation, ester formation or amide formation.

The term "a GLP derivative" is used in the present text to designate a derivative of GLP-2 or an analogue thereof. In the present text, the parent peptide from which such a derivative is formally derived is in some places referred to as the "GLP moiety" of the derivative.

5

In a preferred embodiment, the present invention relates to a GLP-2 derivative having a lipophilic substituent attached to any one amino acid residue.

In another preferred embodiment, the present invention relates to a GLP-2 derivative having
10 a lipophilic substituent attached to any one amino acid residue with the proviso that only if the substituent has an ω -carboxylic acid group or is an alkyl group can it be attached to the N-terminal or C-terminal amino acid residue of the parent peptide.

In another preferred embodiment, the present invention relates to a GLP-2 derivative
15 wherein the lipophilic substituent comprises from 4 to 40 carbon atoms, more preferred from 8 to 25 carbon atoms.

In a further preferred embodiment, the present invention relates to a GLP-2 derivative wherein a lipophilic substituent is attached to an amino acid residue in such a way that a
20 carboxyl group of the lipophilic substituent forms an amide bond with an amino group of the amino acid residue.

In a further preferred embodiment, the present invention relates to a GLP-2 derivative wherein a lipophilic substituent is attached to an amino acid residue in such a way that an
25 amino group of the lipophilic substituent forms an amide bond with a carboxyl group of the amino acid residue.

In a further preferred embodiment, the present invention relates to a GLP-2 derivative wherein a lipophilic substituent is attached to the parent peptide by means of a spacer.

30

In a further preferred embodiment, the present invention relates to a GLP-2 derivative wherein a lipophilic substituent is attached to the parent peptide by means of a spacer which is an unbranched alkane α,ω -dicarboxylic acid group having from 1 to 7 methylene groups,

preferably two methylene groups which spacer forms a bridge between an amino group of the parent peptide and an amino group of the lipophilic substituent.

In a further preferred embodiment, the present invention relates to a GLP-2 derivative
5 wherein a lipophilic substituent is attached to the parent peptide by means of a spacer which is an amino acid residue except Cys, or a dipeptide such as Gly-Lys. In the present text, the expression "a dipeptide such as Gly-Lys" is used to designate a dipeptide wherein the C-terminal amino acid residue is Lys, His or Trp, preferably Lys, and wherein the N-terminal amino acid residue is selected from the group comprising Ala, Arg, Asp, Asn, Gly, Glu, Gln,
10 Ile, Leu, Val, Phe and Pro.

In a further preferred embodiment, the present invention relates to a GLP-2 derivative wherein a lipophilic substituent is attached to the parent peptide by means of a spacer which is an amino acid residue except Cys, or is a dipeptide such as Gly-Lys and wherein a
15 carboxyl group of the parent peptide forms an amide bond with an amino group of a Lys residue or a dipeptide containing a Lys residue, and the other amino group of the Lys residue or a dipeptide containing a Lys residue forms an amide bond with a carboxyl group of the lipophilic substituent.

20 In a further preferred embodiment, the present invention relates to a GLP-2 derivative wherein a lipophilic substituent is attached to the parent peptide by means of a spacer which is an amino acid residue except Cys, or is a dipeptide such as Gly-Lys and wherein an amino group of the parent peptide forms an amide bond with a carboxylic group of the amino acid or dipeptide spacer, and an amino group of the amino acid or dipeptide spacer forms an
25 amide bond with a carboxyl group of the lipophilic substituent.

In a further preferred embodiment, the present invention relates to a GLP-2 derivative wherein a lipophilic substituent is attached to the parent peptide by means of a spacer which is an amino acid residue except Cys, or is a dipeptide such as Gly-Lys and wherein a
30 carboxyl group of the parent peptide forms an amide bond with an amino group of the amino acid or dipeptide spacer, and the carboxyl group of the amino acid or dipeptide spacer forms an amide bond with an amino group of the lipophilic substituent.

In a further preferred embodiment, the present invention relates to a GLP-2 derivative wherein a lipophilic substituent is attached to the parent peptide by means of a spacer which is an amino acid residue except Cys, or is a dipeptide such as Gly-Lys, and wherein a carboxyl group of the parent peptide forms an amide bond with an amino group of Asp or
5 Glu, or a dipeptide containing an Asp or Glu residue, and a carboxyl group of the spacer forms an amide bond with an amino group of the lipophilic substituent.

In a further preferred embodiment, the present invention relates to a GLP-2 derivative having a lipophilic substituent which comprises a partially or completely hydrogenated cyclopentano-
10 phenathrene skeleton.

In a further preferred embodiment, the present invention relates to a GLP-2 derivative having a lipophilic substituent which is a straight-chain or branched alkyl group.

15 In a further preferred embodiment, the present invention relates to a GLP-2 derivative having a lipophilic substituent which is the acyl group of a straight-chain or branched fatty acid.

In a further preferred embodiment, the present invention relates to a GLP-2 derivative having a lipophilic substituent which is an acyl group selected from the group comprising
20 $\text{CH}_3(\text{CH}_2)_n\text{CO}-$, wherein n is 4 to 38, preferably $\text{CH}_3(\text{CH}_2)_6\text{CO}-$, $\text{CH}_3(\text{CH}_2)_8\text{CO}-$, $\text{CH}_3(\text{CH}_2)_{10}\text{CO}-$, $\text{CH}_3(\text{CH}_2)_{12}\text{CO}-$, $\text{CH}_3(\text{CH}_2)_{14}\text{CO}-$, $\text{CH}_3(\text{CH}_2)_{16}\text{CO}-$, $\text{CH}_3(\text{CH}_2)_{18}\text{CO}-$, $\text{CH}_3(\text{CH}_2)_{20}\text{CO}-$ and $\text{CH}_3(\text{CH}_2)_{22}\text{CO}-$.

In a further preferred embodiment, the present invention relates to a GLP-2 derivative having
25 a lipophilic substituent which is an acyl group of a straight-chain or branched alkane α,ω -dicarboxylic acid.

In a further preferred embodiment, the present invention relates to a GLP-2 derivative having a lipophilic substituent which is an acyl group selected from the group comprising
30 $\text{HOOC}(\text{CH}_2)_m\text{CO}-$, wherein m is 4 to 38, preferably $\text{HOOC}(\text{CH}_2)_{14}\text{CO}-$, $\text{HOOC}(\text{CH}_2)_{16}\text{CO}-$, $\text{HOOC}(\text{CH}_2)_{18}\text{CO}-$, $\text{HOOC}(\text{CH}_2)_{20}\text{CO}-$ and $\text{HOOC}(\text{CH}_2)_{22}\text{CO}-$.

In a further preferred embodiment, the present invention relates to a GLP-2 derivative having

a lipophilic substituent which is a group of the formula $\text{CH}_3(\text{CH}_2)_p((\text{CH}_2)_q\text{COOH})\text{CHNH}-\text{CO}(\text{CH}_2)_2\text{CO}-$, wherein p and q are integers and p+q is an integer of from 8 to 40, preferably from 12 to 35.

- 5 In a further preferred embodiment, the present invention relates to a GLP-2 derivative having a lipophilic substituent which is a group of the formula $\text{CH}_3(\text{CH}_2)_r\text{CO}-\text{NHCH}(\text{COOH})(\text{CH}_2)_2\text{CO}-$, wherein r is an integer of from 10 to 24.

In a further preferred embodiment, the present invention relates to a GLP-2 derivative having
10 a lipophilic substituent which is a group of the formula $\text{CH}_3(\text{CH}_2)_s\text{CO}-\text{NHCH}((\text{CH}_2)_2\text{COOH})\text{CO}-$, wherein s is an integer of from 8 to 24.

In a further preferred embodiment, the present invention relates to a GLP-2 derivative having a lipophilic substituent which is a group of the formula $\text{COOH}(\text{CH}_2)_t\text{CO}-$ wherein t is an
15 integer of from 8 to 24.

In a further preferred embodiment, the present invention relates to a GLP-2 derivative having a lipophilic substituent which is a group of the formula $-\text{NHCH}(\text{COOH})(\text{CH}_2)_4\text{NH}-\text{CO}(\text{CH}_2)_u\text{CH}_3$, wherein u is an integer of from 8 to 18.

20

In a further preferred embodiment, the present invention relates to a GLP-2 derivative having a lipophilic substituent which is a group of the formula $-\text{NHCH}(\text{COOH})(\text{CH}_2)_4\text{NH}-\text{COCH}((\text{CH}_2)_2\text{COOH})\text{NH}-\text{CO}(\text{CH}_2)_w\text{CH}_3$, wherein w is an integer of from 10 to 16.

- 25 In a further preferred embodiment, the present invention relates to a GLP-2 derivative having a lipophilic substituent which is a group of the formula $-\text{NHCH}(\text{COOH})(\text{CH}_2)_4\text{NH}-\text{CO}(\text{CH}_2)_2\text{CH}(\text{COOH})\text{NH}-\text{CO}(\text{CH}_2)_x\text{CH}_3$, wherein x is an integer of from 10 to 16.

In a further preferred embodiment, the present invention relates to a GLP-2 derivative having
30 a lipophilic substituent which is a group of the formula $-\text{NHCH}(\text{COOH})(\text{CH}_2)_4\text{NH}-\text{CO}(\text{CH}_2)_2\text{CH}(\text{COOH})\text{NHCO}(\text{CH}_2)_y\text{CH}_3$, wherein y is zero or an integer of from 1 to 22.

In a further preferred embodiment, the present invention relates to a GLP-2 derivative which has one lipophilic substituent.

In a further preferred embodiment, the present invention relates to a GLP-2 derivative which has two lipophilic substituents.

- 5 In a further preferred embodiment, the present invention relates to a GLP-2 derivative in which the C-terminal amino acid residue is present in the form of the amide.

In a further preferred embodiment, the present invention relates to a GLP-2 derivative having a lipophilic substituent which can be negatively charged.

10

In a further preferred embodiment, the present invention relates to a GLP-2 derivative the parent peptide of which is selected from the group comprising GLP-2(1-35) or an analogue thereof.

- 15 In a further preferred embodiment, the present invention relates to a GLP-2 derivative derived from a GLP-2 fragment selected from the group comprising GLP-2(1-30); GLP-2(1-31); GLP-2(1-32); GLP-2(1-33); GLP-2(1-34) and GLP-2(1-35).

- In a further preferred embodiment, the present invention relates to a GLP-2 derivative
20 wherein the designation analogue comprises derivatives wherein a total of up to ten amino acid residues have been exchanged with any α -amino acid residue.

- In a further preferred embodiment, the present invention relates to a derivative of a GLP-2 analogue wherein the designation analogue implies that the parent peptide is human GLP-2
25 wherein a total of up to six, more preferred up to three, amino acid residues have been added, deleted or substituted with other amino acid residues which can be coded for by the genetic code.

- In a further preferred embodiment, the present invention relates to a GLP-2 derivative
30 wherein the parent peptide is selected from the group comprising Lys²⁰GLP-2(1-33) and Lys²⁰Arg³⁰GLP-2(1-33).

In a further preferred embodiment, the present invention relates to a GLP-2 derivative

wherein the parent peptide is Arg³⁰Lys³⁴GLP-2(1-34).

In a further preferred embodiment, the present invention relates to a GLP-2 derivative wherein the parent peptide is selected from the group comprising Arg³⁰Lys³⁵GLP-2(1-35);
 5 Arg^{30,35}Lys²⁰GLP-2(1-35) and Arg³⁵GLP-2(1-35).

In a further preferred embodiment, the present invention relates to a GLP-2 derivative which is selected from the group comprising

- 10 Lys²⁰(N^ε-tetradecanoyl)GLP-2(1-33);
- Lys^{20,30}-bis(N^ε-tetradecanoyl)GLP-2(1-33);
- Lys²⁰(N^ε-tetradecanoyl)Arg³⁰GLP-2(1-33);
- Arg³⁰Lys³⁵(N^ε-tetradecanoyl)GLP-2(1-35);
- Arg^{30,35}Lys²⁰(N^ε-tetradecanoyl)GLP-2(1-35);
- 15 Arg³⁵Lys³⁰(N^ε-tetradecanoyl)GLP-2(1-35);
- Arg³⁰Lys³⁴(N^ε-tetradecanoyl)GLP-2(1-34);
- Lys²⁰(N^ε-(ω-carboxynonadecanoyl))GLP-2(1-33);
- Lys^{20,30}-bis(N^ε-(ω-carboxynonadecanoyl))GLP-2(1-33);
- Lys²⁰(N^ε-(ω-carboxynonadecanoyl))Arg³⁰GLP-2(1-33);
- 20 Arg³⁰Lys³⁵(N^ε-(ω-carboxynonadecanoyl))GLP-2(1-35);
- Arg^{30,35}Lys²⁰(N^ε-(ω-carboxynonadecanoyl))GLP-2(1-35);
- Arg³⁵Lys³⁰(N^ε-(ω-carboxynonadecanoyl))GLP-2(1-35); and
- Arg³⁰Lys³⁴(N^ε-(ω-carboxynonadecanoyl))GLP-2(1-34).

- 25 In a further preferred embodiment, the present invention relates to a pharmaceutical composition comprising a GLP-2 derivative and a pharmaceutically acceptable vehicle or carrier.

- In a further preferred embodiment, the present invention relates to the use of a GLP-2
 30 derivative according to the invention for the preparation of a medicament which has a more protracted action than the parent peptide.

In a further preferred embodiment, the present invention relates to the use of a GLP-2 derivative according to the invention for the preparation of a medicament with protracted effect for the treatment of obesity.

- 5 In a further preferred embodiment, the present invention relates to the use of a GLP-2 derivative according to the invention for the preparation of a medicament with protracted effect for the treatment of small bowel syndrome.

10 DETAILED DESCRIPTION OF THE INVENTION

To obtain a satisfactory protracted profile of action of the GLP-2 derivative, the lipophilic substituent attached to the GLP-2 moiety preferably comprises 4-40 carbon atoms, in particular 8-25 carbon atoms. The lipophilic substituent may be attached to an amino group
15 of the GLP-2 moiety by means of a carboxyl group of the lipophilic substituent which forms an amide bond with an amino group of the amino acid to which it is attached. As an alternative, the lipophilic substituent may be attached to said amino acid in such a way that an amino group of the lipophilic substituent forms an amide bond with a carboxyl group of the amino acid. As a further option, the lipophilic substituent may be linked to the GLP-2 moiety
20 via an ester bond. Formally, the ester can be formed either by reaction between a carboxyl group of the GLP-2 moiety and a hydroxyl group of the substituent-to-be or by reaction between a hydroxyl group of the GLP-2 moiety and a carboxyl group of the substituent-to-be. As a further alternative, the lipophilic substituent can be an alkyl group which is introduced into a primary amino group of the GLP-2 moiety.

25

In one preferred embodiment of the invention, the lipophilic substituent is attached to the GLP-2 moiety by means of a spacer in such a way that a carboxyl group of the spacer forms an amide bond with an amino group of the GLP-2 moiety. Examples of suitable spacers are succinic acid, Lys, Glu or Asp, or a dipeptide such as Gly-Lys. When the spacer is succinic
30 acid, one carboxyl group thereof may form an amide bond with an amino group of the amino acid residue, and the other carboxyl group thereof may form an amide bond with an amino group of the lipophilic substituent. When the spacer is Lys, Glu or Asp, the carboxyl group thereof may form an amide bond with an amino group of the amino acid residue, and the

amino group thereof may form an amide bond with a carboxyl group of the lipophilic substituent. When Lys is used as the spacer, a further spacer may in some instances be inserted between the ϵ -amino group of Lys and the lipophilic substituent. In one preferred embodiment, such a further spacer is succinic acid which forms an amide bond with the ϵ -amino group of Lys and with an amino group present in the lipophilic substituent. In another preferred embodiment such a further spacer is Glu or Asp which forms an amide bond with the ϵ -amino group of Lys and another amide bond with a carboxyl group present in the lipophilic substituent, that is, the lipophilic substituent is a N^ε-acylated lysine residue.

- 10 In another preferred embodiment of the present invention, the lipophilic substituent has a group which can be negatively charged. One preferred group which can be negatively charged is a carboxylic acid group.

The parent peptide can be produced by a method which comprises culturing a host cell containing a DNA sequence encoding the peptide and capable of expressing the peptide in a suitable nutrient medium under conditions permitting the expression of the peptide, after which the resulting peptide is recovered from the culture.

The medium used to culture the cells may be any conventional medium suitable for growing the host cells, such as minimal or complex media containing appropriate supplements. Suitable media are available from commercial suppliers or may be prepared according to published recipes (e.g. in catalogues of the American Type Culture Collection). The peptide produced by the cells may then be recovered from the culture medium by conventional procedures including separating the host cells from the medium by centrifugation or filtration, precipitating the proteinaceous components of the supernatant or filtrate by means of a salt, e.g. ammonium sulphate, purification by a variety of chromatographic procedures, e.g. ion exchange chromatography, gel filtration chromatography, affinity chromatography, or the like, dependent on the type of peptide in question.

- 30 The DNA sequence encoding the parent peptide may suitably be of genomic or cDNA origin, for instance obtained by preparing a genomic or cDNA library and screening for DNA sequences coding for all or part of the peptide by hybridisation using synthetic oligonucleotide probes in accordance with standard techniques (see, for example,

Sambrook, J, Fritsch, EF and Maniatis, T, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York, 1989). The DNA sequence encoding the peptide may also be prepared synthetically by established standard methods, e.g. the phosphoramidite method described by Beaucage and Caruthers, *Tetrahedron Letters* **22** (1981), 1859 - 1869, or the method described by Matthes *et al.*, *EMBO Journal* **3** (1984), 801 - 805. The DNA sequence may also be prepared by polymerase chain reaction using specific primers, for instance as described in US 4,683,202 or Saiki *et al.*, *Science* **239** (1988), 487 - 491.

- 10 The DNA sequence may be inserted into any vector which may conveniently be subjected to recombinant DNA procedures, and the choice of vector will often depend on the host cell into which it is to be introduced. Thus, the vector may be an autonomously replicating vector, *i.e.* a vector which exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, e.g. a plasmid. Alternatively, the vector may be one which, when
15 introduced into a host cell, is integrated into the host cell genome and replicated together with the chromosome(s) into which it has been integrated.

The vector is preferably an expression vector in which the DNA sequence encoding the peptide is operably linked to additional segments required for transcription of the DNA, such
20 as a promoter. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA encoding the peptide of the invention in a variety of host cells are well known in the art, cf. for instance Sambrook *et al.*, *supra*.

25

The DNA sequence encoding the peptide may also, if necessary, be operably connected to a suitable terminator, polyadenylation signals, transcriptional enhancer sequences, and translational enhancer sequences. The recombinant vector of the invention may further comprise a DNA sequence enabling the vector to replicate in the host cell in question.

30

The vector may also comprise a selectable marker, e.g. a gene the product of which complements a defect in the host cell or one which confers resistance to a drug, e.g. ampicillin, kanamycin, tetracyclin, chloramphenicol, neomycin, hygromycin or methotrexate.

To direct a parent peptide of the present invention into the secretory pathway of the host cells, a secretory signal sequence (also known as a leader sequence, prepro sequence or pre sequence) may be provided in the recombinant vector. The secretory signal sequence is
5 joined to the DNA sequence encoding the peptide in the correct reading frame. Secretory signal sequences are commonly positioned 5' to the DNA sequence encoding the peptide. The secretory signal sequence may be that normally associated with the peptide or may be from a gene encoding another secreted protein.

10 The procedures used to ligate the DNA sequences coding for the present peptide, the promoter and optionally the terminator and/or secretory signal sequence, respectively, and to insert them into suitable vectors containing the information necessary for replication, are well known to persons skilled in the art (cf., for instance, Sambrook *et al.*, *supra*).

15 The host cell into which the DNA sequence or the recombinant vector is introduced may be any cell which is capable of producing the present peptide and includes bacteria, yeast, fungi and higher eukaryotic cells. Examples of suitable host cells well known and used in the art are, without limitation, *E. coli*, *Saccharomyces cerevisiae*, or mammalian BHK or CHO cell lines.

20

The GLP-2 derivatives of the invention can be prepared by introducing the lipophilic substituent into the parent GLP-2 or GLP-2 analogue using methods known *per se*, see for example WO 95/07931, the contents of which is hereby incorporated in its entirety by reference.

25

N^ε-acylation of a Lys residue can be carried out by using an activated amide of the acyl group to be introduced as the acylating agent, e.g. the amide with benzotriazole. The acylation is carried out in a polar solvent in the presence of a base.

30

Pharmaceutical compositions

Pharmaceutical compositions containing a GLP-2 derivative according to the present

invention may be administered parenterally to patients in need of such a treatment. Parenteral administration may be performed by subcutaneous, intramuscular or intravenous injection by means of a syringe, optionally a pen-like syringe. Alternatively, parenteral administration can be performed by means of an infusion pump. A further option is a
5 composition which may be a powder or a liquid for the administration of the GLP-2 derivative in the form of a nasal or pulmonal spray. As a still further option, the GLP-2 derivatives of the invention can also be administered transdermally, e.g. from a patch, optionally a iontophoretic patch, or transmucosally, e.g. buccally.

- 10 Pharmaceutical compositions containing a GLP-2 derivative of the present invention may be prepared by conventional techniques, e.g. as described in Remington's Pharmaceutical Sciences, 1985 or in Remington: *The Science and Practice of Pharmacy*, 19th edition, 1995.

Thus, the injectable compositions of the GLP-2 derivative of the invention can be prepared
15 using the conventional techniques of the pharmaceutical industry which involves dissolving and mixing the ingredients as appropriate to give the desired end product.

Thus, according to one procedure, the GLP-2 derivative is dissolved in an amount of water which is somewhat less than the final volume of the composition to be prepared. An isotonic
20 agent, a preservative and a buffer is added as required and the pH value of the solution is adjusted - if necessary - using an acid, e.g. hydrochloric acid, or a base, e.g. aqueous sodium hydroxide as needed. Finally, the volume of the solution is adjusted with water to give the desired concentration of the ingredients.

- 25 Examples of isotonic agents are sodium chloride, mannitol and glycerol.

Examples of preservatives are phenol, m-cresol, methyl p-hydroxybenzoate and benzyl alcohol.

- 30 Examples of suitable buffers are sodium acetate and sodium phosphate.

Further to the above-mentioned components, solutions containing a GLP-2 derivative according to the present invention may also contain a surfactant in order to improve the

solubility and/or the stability of the derivative.

A composition for nasal administration of GLP-2 may, for example, be prepared as described in European Patent No. 272097 (to Novo Nordisk A/S) or in WO 93/18785.

5

The GLP-2 derivatives of this invention can be used in the treatment of various diseases. The particular GLP-2 derivative to be used and the optimal dose level for any patient will depend on the disease to be treated and on a variety of factors including the efficacy of the specific peptide derivative employed, the age, body weight, physical activity, and diet of the patient, on a possible combination with other drugs, and on the severity of the case. It is recommended that the dosage of the GLP-2 derivative of this invention be determined for each individual patient by those skilled in the art in a similar way as for known parent peptides.

15 The pharmacological properties of the compounds of the invention can be tested e.g. as described in our International Patent Application No. PCT/DK97/00086 the contents of which is hereby incorporated in its entirety by reference.

The present invention is further illustrated by the following examples which, however, are not to be construed as limiting the scope of protection. The features disclosed in the foregoing description and in the following examples may, both separately and in any combination thereof, be material for realizing the invention in diverse forms thereof.

25

EXAMPLES

The following acronyms for commercially available chemicals are used:

NMP :	N-Methyl-2-pyrrolidone.
30 EDPA :	N-Ethyl-N,N-diisopropylamine.
TFA :	Trifluoroacetic acid.
Myr-ONSu:	Tetradecanoic acid 2,5-dioxopyrrolidin-1-yl ester.

Abbreviations:

PDMS: Plasma Desorption Mass Spectrometry

HPLC: High Performance Liquid Chromatography

5 amu: atomic mass units

EXAMPLE 1

Synthesis of Lys³⁰ (N^E-tetradecanoyl) hGLP-2.

10

A mixture of hGLP-2 (10.0 mg, 2.7 μ mol), EDPA (9.6 mg, 74.3 μ mol), NMP (210 μ l) and water (100 μ l) was gently shaken for 15 min. at room temperature. To the resulting mixture was added a solution of Myr-ONSu (21.5 mg, 6.6 μ mol) in NMP (32 μ l). The reaction mixture was gently shaken for 30 min. at room temperature, and an additional amount of a solution of Myr-ONSu (14.4 mg, 4.4 μ mol) in NMP (22 μ l). The resulting mixture was gently shaken for 15 min. at room temperature. The reaction was quenched by the addition of a solution of glycine (4.5 mg, 4.5 μ mol) in 50% aqueous ethanol (451 μ l). The reaction mixture was purified by column chromatography using a cyanopropyl column (Zorbax 300SB-CN) and a standard acetonitrile/TFA system. The column was heated to 65°C and the acetonitrile gradient was 0-100% in 60 minutes. The title compound (5.0 mg, 47 %) was isolated from the eluate.

15

20

CLAIMS

1. A GLP-2 derivative comprising a lipophilic substituent attached to any one amino acid residue.
5
2. A GLP-2 derivative according to claim 1 with the proviso that only if the substituent has an ω -carboxylic acid group or is an alkyl group can it be attached to the N-terminal or C-terminal amino acid residue of the parent peptide.
- 10 3. A GLP-2 derivative according to claim 1 or 2, wherein the lipophilic substituent comprises from 4 to 40 carbon atoms, more preferred from 8 to 25.
4. A GLP-2 derivative according to anyone of the preceding claims, wherein said lipophilic substituent is attached to said amino acid in such a way that a carboxyl group of the
15 lipophilic substituent forms an amide bond with an amino group of the amino acid.
5. A GLP-2 derivative according to anyone of the claims 1-3, wherein said lipophilic substituent is attached to said amino acid in such a way that an amino group of the lipophilic substituent forms an amide bond with a carboxyl group of the amino acid.
20
6. A GLP-2 derivative according to anyone of the preceding claims, wherein the lipophilic substituent is attached to the parent peptide by means of a spacer.
7. A GLP-2 derivative according to claim 6, wherein the spacer is an unbranched alkane
25 α,ω -dicarboxylic acid group having from 1 to 7 methylene groups, preferably two methylene groups which form a bridge between an amino group of the parent peptide and an amino group of the lipophilic substituent.
8. A GLP-2 derivative according to claim 6, wherein the spacer is an amino acid residue
30 except Cys, or a dipeptide such as Gly-Lys.
9. A GLP-2 derivative according to claim 8, wherein a carboxyl group of the parent peptide forms an amide bond with an amino group of Lys or a dipeptide containing a Lys residue,

and the other amino group of the Lys or a dipeptide containing a Lys residue forms an amide bond with a carboxyl group of the lipophilic substituent.

- 10.A GLP-2 derivative according to claim 8, wherein an amino group of the parent peptide
5 forms an amide bond with a carboxylic group of the amino acid or dipeptide spacer, and
an amino group of the amino acid or dipeptide spacer forms an amide bond with a
carboxyl group of the lipophilic substituent.
- 11.A GLP-2 derivative according to claim 8, wherein a carboxyl group of the parent peptide
10 forms an amide bond with an amino group of the amino acid or dipeptide spacer, and the
carboxyl group of the amino acid or dipeptide spacer forms an amide bond with an amino
group of the lipophilic substituent.
- 12.A GLP-2 derivative according to claim 8, wherein a carboxyl group of the parent peptide
15 forms an amide bond with an amino group of Asp or Glu, or a dipeptide containing an Asp
or Glu residue, and a carboxyl group of the spacer forms an amide bond with an amino
group of the lipophilic substituent.
- 13.A GLP-2 derivative according to anyone of the preceding claims, wherein the lipophilic
20 substituent comprises a partially or completely hydrogenated cyclopentanophenathrene
skeleton.
- 14.A GLP-2 derivative according to any of claims 1-12, wherein the lipophilic substituent is
an straight-chain or branched alkyl group.
- 25 15.A GLP-2 derivative according to any of claims 1-12, wherein the lipophilic substituent is
the acyl group of a straight-chain or branched fatty acid.
- 16.A GLP-2 derivative according to claim 15 wherein the acyl group is selected from the
30 group comprising $\text{CH}_3(\text{CH}_2)_n\text{CO}-$, wherein n is 4 to 38, preferably $\text{CH}_3(\text{CH}_2)_6\text{CO}-$,
 $\text{CH}_3(\text{CH}_2)_8\text{CO}-$, $\text{CH}_3(\text{CH}_2)_{10}\text{CO}-$, $\text{CH}_3(\text{CH}_2)_{12}\text{CO}-$, $\text{CH}_3(\text{CH}_2)_{14}\text{CO}-$, $\text{CH}_3(\text{CH}_2)_{16}\text{CO}-$,
 $\text{CH}_3(\text{CH}_2)_{18}\text{CO}-$, $\text{CH}_3(\text{CH}_2)_{20}\text{CO}-$ and $\text{CH}_3(\text{CH}_2)_{22}\text{CO}-$.

- 17.A GLP-2 derivative according to any of claims 1-12, wherein the lipophilic substituent is an acyl group of a straight-chain or branched alkane α,ω -dicarboxylic acid.
- 18.A GLP-2 derivative according to claim 17 wherein the acyl group is selected from the group comprising $\text{HOOC}(\text{CH}_2)_m\text{CO-}$, wherein m is 4 to 38, preferably $\text{HOOC}(\text{CH}_2)_{14}\text{CO-}$, $\text{HOOC}(\text{CH}_2)_{16}\text{CO-}$, $\text{HOOC}(\text{CH}_2)_{18}\text{CO-}$, $\text{HOOC}(\text{CH}_2)_{20}\text{CO-}$ and $\text{HOOC}(\text{CH}_2)_{22}\text{CO-}$.
- 19.A GLP-2 derivative according to any of claims 1-12, wherein the lipophilic substituent is a group of the formula $\text{CH}_3(\text{CH}_2)_p((\text{CH}_2)_q\text{COOH})\text{CHNH-CO}(\text{CH}_2)_2\text{CO-}$, wherein p and q are integers and p+q is an integer of from 8 to 40, preferably from 12 to 35.
- 20.A GLP-2 derivative according to any of claims 1-12, wherein the lipophilic substituent is a group of the formula $\text{CH}_3(\text{CH}_2)_r\text{CO-NHCH}(\text{COOH})(\text{CH}_2)_2\text{CO-}$, wherein r is an integer of from 10 to 24.
- 21.A GLP-2 derivative according to any of claims 1-12, wherein the lipophilic substituent is a group of the formula $\text{CH}_3(\text{CH}_2)_s\text{CO-NHCH}((\text{CH}_2)_2\text{COOH})\text{CO-}$, wherein s is an integer of from 8 to 24.
- 22.A GLP-2 derivative according to any of claims 1-12, wherein the lipophilic substituent is a group of the formula $\text{COOH}(\text{CH}_2)_t\text{CO-}$ wherein t is an integer of from 8 to 24.
- 23.A GLP-2 derivative according to any of claims 1-12, wherein the lipophilic substituent is a group of the formula $-\text{NHCH}(\text{COOH})(\text{CH}_2)_4\text{NH-CO}(\text{CH}_2)_u\text{CH}_3$, wherein u is an integer of from 8 to 18.
- 24.A GLP-2 derivative according to any of claims 1-12, wherein the lipophilic substituent is a group of the formula $-\text{NHCH}(\text{COOH})(\text{CH}_2)_4\text{NH-COCH}((\text{CH}_2)_2\text{COOH})\text{NH-CO}(\text{CH}_2)_w\text{CH}_3$, wherein w is an integer of from 10 to 16.
- 25.A GLP-2 derivative according to any of claims 1-12, wherein the lipophilic substituent is a group of the formula $-\text{NHCH}(\text{COOH})(\text{CH}_2)_4\text{NH-CO}(\text{CH}_2)_2\text{CH}(\text{COOH})\text{NH-CO}(\text{CH}_2)_x\text{CH}_3$, wherein x is an integer of from 10 to 16.

26.A GLP-2 derivative according to any of claims 1-12, wherein the lipophilic substituent is a group of the formula $\text{-NHCH(COOH)(CH}_2\text{)}_4\text{NH-CO(CH}_2\text{)}_2\text{CH(COOH)NHCO(CH}_2\text{)}_y\text{CH}_3$, wherein y is zero or an integer of from 1 to 22.

5

27.A GLP-2 derivative according to any of the preceding claims which has one lipophilic substituent.

28.A GLP-2 derivative according to any one of claims 1-26 which has two lipophilic substituents.

10

29.A GLP-2 derivative according anyone of the preceding claims, wherein the parent peptide is selected from the group comprising GLP-2(1-30); GLP-2(1-31); GLP-2(1-32); GLP-2(1-33); GLP-2(1-34) and GLP-2(1-35) or an analogue or a fragment thereof.

15

30.A GLP-2 derivative according to claim 29, wherein the parent peptide is selected from the group comprising GLP-2(1-35) or an analogue or a fragment thereof.

31.A GLP-2 derivative according to any of the claims 29 and 30 wherein the designation analogue comprises derivatives wherein a total of up to ten amino acid residues have been exchanged with any α -amino acid residue.

20

32.A GLP-2 derivative according to any of the preceding claims wherein the parent peptide is selected from the group comprising $\text{Lys}^{20}\text{GLP-2(1-33)}$; $\text{Lys}^{20}\text{Arg}^{30}\text{GLP-2(1-33)}$; $\text{Arg}^{30}\text{Lys}^{35}\text{GLP-2(1-35)}$; $\text{Arg}^{30,35}\text{Lys}^{20}\text{GLP-2(1-35)}$; $\text{Arg}^{35}\text{GLP-2(1-35)}$; $\text{Arg}^{30}\text{Lys}^{34}\text{GLP-2(1-34)}$.

25

33.A GLP-2 derivative according to anyone of the preceding claims, which is selected from the group consisting of

30

$\text{Lys}^{20}(\text{N}^{\epsilon}\text{-tetradecanoyl})\text{GLP-2(1-33)}$;

$\text{Lys}^{20,30}\text{-bis}(\text{N}^{\epsilon}\text{-tetradecanoyl})\text{GLP-2(1-33)}$;

$\text{Lys}^{20}(\text{N}^{\epsilon}\text{-tetradecanoyl})\text{Arg}^{30}\text{GLP-2(1-33)}$;

- Arg³⁰Lys³⁵(N^ε-tetradecanoyl)GLP-2(1-35);
 Arg^{30,35}Lys²⁰(N^ε-tetradecanoyl)GLP-2(1-35);
 Arg³⁵Lys³⁰(N^ε-tetradecanoyl)GLP-2(1-35);
 Arg³⁰Lys³⁴(N^ε-tetradecanoyl)GLP-2(1-34);
 5 Lys²⁰(N^ε-(ω-carboxynonadecanoyl))GLP-2(1-33);
 Lys^{20,30}-bis(N^ε-(ω-carboxynonadecanoyl))GLP-2(1-33);
 Lys²⁰(N^ε-(ω-carboxynonadecanoyl))Arg³⁰GLP-2(1-33);
 Arg³⁰Lys³⁵(N^ε-(ω-carboxynonadecanoyl))GLP-2(1-35);
 Arg^{30,35}Lys²⁰(N^ε-(ω-carboxynonadecanoyl))GLP-2(1-35);
 10 Arg³⁵Lys³⁰(N^ε-(ω-carboxynonadecanoyl))GLP-2(1-35); and
 Arg³⁰Lys³⁴(N^ε-(ω-carboxynonadecanoyl))GLP-2(1-34).

- 34.A pharmaceutical composition comprising a GLP-2 derivative according to any of the preceding claims and a pharmaceutically acceptable vehicle or carrier.
- 15 35.Use of a GLP-2 derivative according to any of the claims 1-33 for the preparation of a medicament.
- 36.Use of a GLP-2 derivative according to any of the claims 1-33 for the preparation of a
 20 medicament with protracted effect.
- 37.Use of a GLP-2 derivative according to any of claims 1-33 for the preparation of a medicament with protracted effect for the treatment of obesity.
- 25 38.Use of a GLP-2 derivative according to any of claims 1-33 for the preparation of a medicament with protracted effect for the treatment of small bowel syndrome.
- 39.A method of treating obesity in a patient in need of such a treatment, comprising administering to the patient a therapeutically effective amount of a GLP-2 derivative
 30 according to any one of the claims 1-33 together with a pharmaceutically acceptable carrier.

40.A method of treating small bowel syndrome in a patient in need of such a treatment, comprising administering to the patient a therapeutically effective amount of a GLP-2 derivative according to any one of the claims 1-33 together with a pharmaceutically acceptable carrier.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 97/00360

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07K 14/605, A61K 38/26

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07K, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

REG, CAPLUS, WPI, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9111457 A1 (BUCKLEY, DOUGLAS), 8 August 1991 (08.08.91), see claims --	1-2
X	US 5512549 A (VICTOR J. CHEN ET AL), 30 April 1996 (30.04.96), see table 1, line 65 --	1-38
P,A	WO 9632414 A1 (ONTARIO INC.), 17 October 1996 (17.10.96), see page 19, claims -- -----	34-38

☐ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "B" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search

10 December 1997

Date of mailing of the international search report

11-12-1997

Name and mailing address of the ISA/
Swedish Patent Office
Box 5055, S-102 42 STOCKHOLM
Facsimile No. +46 8 666 02 86

Authorized officer

Carolina Gómez Lagerlöf
Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 97/00360

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 39-40
because they relate to subject matter not required to be searched by this Authority, namely:

See PCT Rule 39.1(iv): Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐

The additional search fees were accompanied by the applicant's protest.

☐

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 97/00360

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9111457 A1	08/08/91	CA 2073856 A EP 0512042 A US 5545618 A	25/07/91 11/11/92 13/08/96
US 5512549 A	30/04/96	AU 3432295 A BR 9504452 A CA 2160753 A CN 1129224 A CZ 9502666 A EP 0708179 A FI 954941 A HU 73413 A HU 9503001 D IL 115583 D JP 8245696 A NO 954055 A PL 310961 A	02/05/96 20/05/97 19/04/96 21/08/96 15/05/96 24/04/96 19/04/96 29/07/96 00/00/00 00/00/00 24/09/96 19/04/96 29/04/96
WO 9632414 A1	17/10/96	AU 5265896 A	30/10/96